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ORGANIZATION AND METHOD OF FIGHTING ANTHRAX

- USSR -

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ORGANIZATION AND METHOD OF FIGHTING ANTHRAX

- USSR -

[Following is a translation of selections from the Russian-language book Sibirskaya yazva; sbornik organizatsionnykh i metodicheskikh materialov (Anthrax; Collected Data on Organization and Method) edited by B. M. Pastukhov and compiled by E. N. Shlyakhov and V. A. Sinodskaya, Moscow, State Publishing House for Medical Literature, 1962, pages 3-4, 40-68, 74-89.]

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INTRODUCTION

The grandiose program set forth in the seven-year plan for the development of the Soviet national economy and the decree of the Central Committee of the CPSU and the Council of Ministers of the USSR outlines many diverse targets for the Soviet public health service. Of considerable importance among these targets is that of working out sanitary-antiepidemic measures aimed at sharply reducing and eventually eliminating a number of infectious diseases which afflict people in our country.

The problem of bringing about a sharp reduction in the incidence of anthrax morbidity during the next few years is at once most timely and realizable.

If this is to be accomplished, we must have complete coordination of effort among health service organs and those of other agencies, first and foremost, of the Ministry of Agriculture. There must be purposeful and effective leadership on the part of medical and agricultural personnel.

We have developed instructions and rules pertaining to the epidemiology, diagnostics, treatment, and chiefly, the prophylaxis of anthrax in humans and animals. These materials have been published by the ministries of health, agriculture, railways, as well as other agencies.

As yet, these materials have not been assembled, and this has to a considerable extent made it difficult for practical workers to make effective use of them.

In view of the positive results which have accrued from the publication of collected data on a number of infectious diseases, in particular, on zoonotic diseases (brucellosis, tularemia, and others), we deemed it advisable to publish a similar collection of data on anthrax.

The present collection contains data on the most important medical data on methods, as well as important veterinary-sanitary instructions and directions of interest to medical personnel.

The collection is intended for sanitation-epidemiology specialists and physicians in treatment and prophylactic clinics: epidemiologists, microbiologists, infectious diseases specialists, and other middle-echelon medical personnel in these clinics.

The compilers of this collection welcome critical comments and suggestions for improving the next edition.

INSTRUCTIONS ON THE PROCEDURE FOR STORING, HANDLING, AND DISPENSING PATHOGENIC BACTERIA, VIRUSES, AND OTHER MICROBES, AS WELL AS BACTERIAL TOXINS AND POISONS OF ANIMAL ORIGIN.

(Approved 19 March 1954 by the Main Sanitation-Antiepidemic Administration of the Ministry of Health USSR)

I. General

1. A single uniform and obligatory procedure for storing, handling and dispensing operations is established for all scientific research and treatment institutes, sanitation-epidemiological stations and laboratories which work with bacterial cultures, toxins, and poisons of animal origin. This procedure must ensure work safety in laboratories and preclude the possibility of dispensing cultures, toxins, and poisons beyond the walls of these installations without special permission.

2. The various bacteria, viruses and other microbes, and also toxins, are broken down into four different groups according to pathogenic action; a definite procedure for storage, dispensing and handling in the laboratory is established for each group.

Group I. Agents of highly contagious diseases (especially dangerous infections): smallpox, glanders, tularemia, brucellosis, anthrax, psittacosis, poliomyletis, ornithosis, encephalitis, rabies, hemorrhagic fever, rickettsiosis (exanthematous fever, tsutsugamushi, Rocky Mountain Fever, Q fever).

3. Institutions continuously working with pathogenic bacteria,

viruses and other Group I microbes are permitted to maintain collections of strains for the purpose of research, production and diagnosis.

5. Institutes and other installations authorized to work with Group I cultures are required to organize a special laboratory (museum) for live cultures and to appoint a person to head the laboratory (museum), who will also be in charge of documentation relating to incoming and outgoing cultures.

6. All strains in the possession of an installation are to be concentrated in the laboratory (museum).

8. Heads of live culture laboratories (museums) must be bacteriologists-physicians with a minimum of three years experience. In central installations, heads of live culture laboratories are to be approved by the Main Sanitation-Antiepidemic Administration of the Ministry of Health USSR; in other installations, approval will be given, based on the recommendation of institution directors, by the sanitation-antiepidemic administration of the ministry of health of a given union republic.

In small laboratories, staffed by one or two physicians, the responsibility for maintaining the collection of cultures is assigned to the head of the installation (laboratory).

9. Records are to be kept on all existing strains and all new strains received, together with all data (subculturing, passage, dispensation, destruction) pertaining to the handling and disposition of the strains; these records are to be kept in specially numbered and

bound registers, which will be closed with the seal of the installation or of the first section. For this purpose, the laboratories and museums working with live cultures must keep the following records:

a. Inventory Register of Museum Cultures (Form No. 1). All existing cultures and all cultures received, after they have been identified, are to be entered in this register under a given number, together with the name of the microbe or virus. Every Group I strain kept in the museum or laboratory, for one reason or another, must be registered in the Inventory Register.

b. Register of Isolated Cultures (Form No. 2). Strains isolated in the process of diagnosis, which will not be kept in the laboratory, are to be registered in the Register of Isolated Cultures.

c. Register of Disposition of Cultures (Form No. 3). The maintaining of this register is obligatory for laboratories having strains recorded in the Inventory Register of Museum Cultures.

d. Register of the Movement of Infectious Material (Form No. 4). The keeping of this register is obligatory for all laboratories engaged in bacteriological work.

e. Register for the Dispensing of Cultures and Toxins (Form No. 6). This register is for the recording of cultures and toxins dispensed by the laboratory within given installation, as well as to outside organizations.

f. Upon issuing cultures to other installations, the issuing organization must fill out documentation to accompany the cultures

(Form No. 7).

10. All registers and other documentation relating to the cultures (receiving, destruction, authorization to obtain cultures) are to be kept locked in a safe, strong box, or cabinet under the care of those persons responsible for these cultures. These registers are to be used only by persons authorized to work with the given cultures.

11. Test tubes and ampules containing the strains should be clearly labeled with indelible ink and sealed at the top with a thin layer of parafin; a gummed label and rubber stopper may also be used. In any case, they must bear the name of the bacteria, the strain's number in the inventory register and dates of subculturing. Working cultures must also be clearly labeled.

12. In the subculturing of museum cultures, the following conditions must be fulfilled: a) only one species (strain) of microbes may be on the work table at one time; b) museum cultures are to be grown with the aid of special thermostats; c) passage of museum virus strains is to be carried out in areas specially set aside for this purpose.

13. After subculturing new museum strains in new media, the cultures used for the subculturing are destroyed and this destruction noted in the proper registers. The destruction of old cultures is performed upon the establishment of the purity and correctness of the subculturing and establishing the correspondence of inscriptions on the new and old cultures, etc. Sealed duplicate test tubes containing

cultures may be kept in the museum for the purpose of scientific observation; if so, this must be duly noted in the proper register.

14. Museum culture strains are to be kept in a refrigerator or in a fire-proof cabinet. The strains are to be kept in sealed ampules which have been dried in a vacuum. Should the drying operation be impossible, the keeping of strains in agar in sealed test tubes inside a refrigerator is permitted; organs from diseased animals are to be kept in the refrigerator in test tubes containing 50 percent glycerine.

15. All refrigerators and cabinets containing cultures must be kept locked; at the end of the day, they are to be sealed with sealing wax or mastic cement. Keys to the locks, and the sealing materials are kept by the head of the laboratory. It is categorically forbidden to leave cultures on the tables or in unlocked and unsealed places at the close of the working day. Doors to the rooms containing the live cultures are to be locked and sealed with sealing wax. Bars should be installed on the windows of the laboratories where Group I cultures are used or stored. Keys to laboratories and to the building are to be given to the duty officer, who is required to sign for them. (These keys are kept in a special cabinet.) Buildings housing pathogenic cultures should be guarded.

16. When it becomes necessary to interrupt scientific work (leave, business trip, or other reasons), the collection of cultures and the pertinent registers are transferred to the live cultures

laboratory for safe keeping.

Remarks: 1. The temporary transfer of cultures for storage in the live cultures laboratory is effected by a notice, written by a member of the staff, and approved by the director or the deputy director. Cultures to be stored must be in sealed ampules.

2. Cultures stored in the live cultures laboratory must be stored separately in a sealed case. Subculturing of cultures stored in the museum of cultures will be done upon the written request of the person presenting the cultures for storage, with the consent of the director.

3. In research institutes with a large volume of work in the field of microbiology, cultures assigned to staff members may be left in the laboratory and kept in a sealed, fire-proof safe or refrigerator.

17. All cultures of bacteria and viruses, and also diseased animals, which the directors of departments have received from one or another laboratory, are entered in the inventory register (forms No. 1 and 2) and in the register for the disposition of infectious material (forms No. 3 and 4) and are stored in the department. Issuance of subcultures of these strains to individual staff members is recorded on forms No. 3 or 4. The issuance must be accompanied by a signature.

18. The dispensing of strains from the live cultures laboratory (museum) to a department or laboratory of the same installation is done with the written consent of the head of the department or the head of the installation (a physician) or his deputy for scientific work.

19. The transfer of cultures from one department to another

department in the same installation is made with the consent of the head of the installation. The transfer is made through the live cultures laboratory (museum), and the transfer is entered in the register for dispensing of cultures (Form No. 6). It is noted whether the live cultures laboratory checked the culture to be transferred. A tag must be attached to the culture.

All culture registers in the departments and laboratories must be numbered and bound and sealed with the seal of the special section or installation.

20. When the bacteriological departments of antiplague institutes, sanitation-epidemiological stations and other installations isolate, in the course of their research work, cultures of Group I microbes, these are to be identified and studied. The identified culture must be registered in the inventory register (Form No. 1), and its disposition recorded in the disposition register (Form No. 3). Isolated strains not to be stored are registered on Form No. 2 and destroyed (Form No. 9). Where a strain is to be stored for an extended period, it is presented to the live cultures laboratory, with the consent of the director of the installation.

21. When Group I cultures are isolated by installations not authorized to store them, upon receiving written authorization from higher authority, they either destroy the culture and make a record of the destruction, or ship the culture to an installation authorized to store the culture.

22. Every staff member, including department and laboratory chiefs, who receives Group I cultures, or animals infected with these cultures, to work on, is required to keep a record of the disposition and movement of these cultures during the working day, if he keeps these strains for work or subculturing. He must also hatch additional bacteria for the next day's work. The record is kept on forms 3 and 4.

23. The museum where the strains are to be stored separately should be outfitted with iron boxes and metal cases. These cases or boxes are to be stored in metal fire-proof cabinets or refrigerators.

24. Cultures used for preparation of live vaccines are stored in separate cabinets. Following the subculturing, the cultures are placed in thermostatically-controlled enclosures allocated only for the time needed to grow these strains. The preparation of vaccines from these strains is done according to special instructions.

25. Every laboratory which works with bacteria of any of the four groups is required to have metal waterproof tanks with lids for the collection and sterilization of materials (receptacles, animal corpses, etc.). Tanks containing infectious materials should be sealed.

26. All cultures -- museum and working -- to be destroyed are given to a specially appointed staff member for destruction by autoclave. A note to this effect is entered on Form No. 1 or 2. The corpses and organs of infected animals, food leftovers, and bedding are placed in a receptacle containing a disinfectant, and is

presented — in exchange for a receipt -- for destruction by burning or autoclave, this fact being noted in the register for infectious materials.

27. Upon transferring the affairs of a department, laboratory, or a section, because of the discharge or leave taken by a person responsible for storage, all documents (registers, certificates, directives, etc.) relating to the registering and storing of cultures, and also all seals, are given to the person's replacement, together with the inventory statement and the numbers of documents transferred, and a statement verifying the existence of cultures listed in the registers.

28. Heads of departments and laboratories are charged with seeing that these instructions are carried out. A check should be made not less than once every four months.

29. These instructions must be carried out by all institutes, installations, and laboratories having to do with pathogenic cultures, viruses, toxins and poisons. In the event these instructions are violated, the violators may be made to answer for it.

30. All previously issued regulations governing the procedure for storing and dispensing cultures are rescinded with the publication of the present instructions.

31. The responsibility for organizing the registration of live cultures in institutes and laboratories (departments) rests with the director of the installation and the head of the live cultures

laboratory (museum), as well as with chiefs of departments; in installations of the sanitation-epidemiological service, responsibility rests with the chief physicians and with the heads of the live cultures laboratories (museums).

III. Rules for Working with Group I Agents

32. All work with Group I cultures must be carried out exclusively in installations having special authorization from the Main Sanitation-Antiepidemic Administration of the Ministry of Health USSR. Such work can only be performed in specially outfitted laboratories which provide full isolation and safety for the surrounding populace, as well as providing a full measure of safety for the personnel employed in the laboratories.

33. All work, performed by the institutes, antiplague and other special installations and laboratories, connected with cultures of pathogenic microbes and viruses of Group I, must be carried out in strict accordance with the special instruction "The Work Regime", confirmed by the Ministry of Health of the USSR.

34. The list of installations authorized to work with Group I live cultures is approved by the Main Sanitation-Antiepidemic Administration of the Ministry of Health of the USSR. The list of personnel authorized to work with Group I live cultures is confirmed by a directive issued by the installation's director.

Note: Only physicians (medical and veterinary) and biologists have the right to dispense and receive Group I cultures for use

in laboratories, institutes, and other installations.

35. Each staff member who works with Group I cultures is assigned his own thermostatic enclosure, a cabinet or sealable box, or a section of a cupboard and, if possible, an individual icebox.

36. When work with a strain is finished, or when the strain is no longer needed, the staff worker who has charge of the given strain, with the consent of the department head, must destroy all subcultures of the given strain in the presence of a commission made up of department staff members. The destruction is registered on forms No. 8 and 9, and a note is made in the inventory register of museum cultures or in the register of isolated cultures (form No. 1 and 2).

37. The transfer of cultures from one department to another within the same building is effected in special closed metal tins. When the cultures are carried through the courtyard, upon their transfer from one building to another, the cultures are carried in sealed tins.

The Procedure for Dispensing Cultures, Pathogenic Bacteria, and Toxins (Beyond the Bounds of the Installation).

49. The dispensing of Group I bacteria and viruses from institutes and other installations is carried out only with the authorization of the director of the State Control Institute for Medical Biological Preparations im. L. A. Tarasevich.

In order to obtain Group I microbic cultures, the installation director must submit, in duplicate, a request bearing a stamp and a

circular seal, to the director of the State Control Institute for Medical Biological Preparations im. L. A. Tarasevich.

Group I cultures are released to couriers upon presentation of the approved request and official credentials bearing a stamp and circular seal; the courier must also present his passport or other means of identification.

Note: The signature of the courier to whom the culture is entrusted must be certified by the director of the installation.

50. Upon receiving the culture, the installation must immediately dispatch a written statement, attesting to the receipt.

The staff member who is responsible for registering cultures must note this statement on Form No. 6.

51. Group I bacteria may be released beyond the confines of a given installation only in sound nutrient media or in dried form, excepting those cases providing for the transport of cultures in glycerine.

Test tubes containing cultures must be sealed and placed in special metal cases. These cases are then placed in wooden boxes.

The boxes containing the cultures must be sealed. Form No. 7 accompanies the dispensed cultures.

52. Materials intended for virusological research (individual animal and human organs) are transported by the courier in test tubes or tins containing a 50 percent solution of glycerine enclosed in rubber waterproofing material; the latter is placed, together with ice, in a thermos which is then sealed.

FORM NO. 1

INVENTORY REGISTER OF MUSEUM CULTURES

Number	Name of microbe in Latin transcription	Special name	Number of strain	Time of isolation	Place of isolation (oblast, city, settlement)	In whom or what isolated. Number of case history; material	Strain received from...; Number of accompanying document	Date received	Strain characteristics	Destruction or transfer	Remarks
1	2	3	4	5	6	7	8	9	10	11	12

Note: Movement of museum cultures is recorded in the Register of Disposition of Museum Cultures (Form No. 3).

FORM NO. 2

REGISTER OF ISOLATED CULTURES

Number	Name of culture	Time of isolation	In whom or what isolated; Number of case history	In what material isolated	Strain characteristics (morphology, biochemical activity, serological features)	When and where culture sent for analysis, (Number in inventory register) or destruction (date of destruction and number of documentation pertaining to destruction)	Signature of person responsible for keeping the register

Note: The movement of isolated cultures prior to their destruction or transfer to the museum is recorded in the Register of Movement of Infectious Material (form No. 4).

FORM NO. 3

REGISTER OF DISPOSITION OF MUSEUM CULTURES

1	Name of microbe in Latin transcription		
2	Number of strain		
3	Number in inventory register		
	Movement of cultures		
4	Date		
5	Beakers (vials, etc.) containing cultures	Number at start of working day	
6		Number subcultured or received	
7		Number destroyed	
		Transferred	
8		Where dispatched	
9		Number	
10		Receipt	
11		Number left at end of day	
	Ampules containing dried cultures		
12		Number at start of working day	
13		Number subcultured or received	
14		Number destroyed	
		Transferred	
15		Where dispatched	
16		Number	
17		Receipt	
18		Number left at end of day	
19	Signature of head of museum		

Note: At least two pages are used for each strain; if the laboratory does not have dried cultures, items 12-18 may be omitted. Entries are made in the register only on the days the cultures are moved. Multiplication of cultures for research is noted in register. Data on receptacles bearing the cultures is recorded in 5-11.

FORM NO. 4

REGISTER OF MOVEMENT OF INFECTIOUS MATERIAL (USED IN CURRENT DIAGNOSTIC OR EXPERIMENTAL WORK)

			Count of beakers, dishes, vials, etc. containing cultures			Count of beakers, etc., containing animal organs			Count of diseased animals by type				Count of dried cultures					
Date	Name of culture or analysis	Number at start of working day	Number subcultured or received	Destroyed or transferred	Remainder at end of day	Number at start of day	Number received	Destroyed or transferred	Remainder at end of day	Number of animals at start of working day	Number infected	Died or destroyed	Number of animals left at end of day	Number of ampules at start of day	Number received from dryer	Destroyed or transferred; quantity and destination	Receipt	Number remaining at end of day	Remarks
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20

Note: If the laboratory is not working with a given material (animals or dried cultures), it may omit corresponding items in the register. When work is done on large infected embryos or other matter not covered by the register, the necessary items must be added. Transfer of cultures to the museum (or to other staff worker) is recorded in item 5 or 17; the name of the recipient, the quantity (beakers, vials) is noted. Upon receiving cultures from the museum (or from staff member), items 4 or 8 must be filled out. The various vessels containing cultures or organs are noted in items 3-19.

FORM NO. 6

REGISTER FOR THE DISPENSING OF CULTURES (OUTSIDE THE INSTALLATION AS WELL AS BETWEEN DEPARTMENTS OF THE SAME INSTALLATION)

Number	Date request was received	Requesting agency (where culture was sent	Person authorizing dispensing of shipment of material, number and date of authorizing document	Name of culture. Number of strain.	Quantity of cultures released. Indicate container and packaging	Date culture was dispensed	By whom dispensed	Received by... Date and number of credentials. Number of passport.	Receipt	Acknowledgement of receipt of shipment from receiving installation No.____	Remarks
1	2	3	4	5	6	7	8	9	10	11	12

53. The shipment of Group I cultures from one installation to another must be authorized by the director of a given installation. The shipment (by motor vehicle, airplane or train (in a separate compartment) must be accompanied by two persons, one of whom must be a physician.

54. The shipment of cultures is opened in the live cultures laboratory (musemm) in the presence of a commission which includes the director of the receiving installation, the head of the museum, the head of the laboratory or department or section, and the physician working with the culture in question.

Form No. 10 is filled out upon the receipt of the culture.

The arrival of the culture is registered in the inventory register and in the register for disposition of cultures (Form No. 1 and 3).

FORM NO. 7

Certificate

Species of microbe . . . . . .
Strain number . . . . . .
Special name of strain . . . . . .
Date isolated . . . . . .
Where isolated . . . . . .
From where received . . . . . .
Data received . . . . . .
Date of last examination of strain's properties . . . . . .

The Strain's Characteristics

1. Morphological features (cellular form, motion, sporulation, flagella, capsules, etc.) and tinctorial properties.
2. Cultural features: particulars concerning growth in various nutrient media:
   a. in implanting culture on the surface of a firm medium (indicate medium and type of receptacle -- beaker, dish, etc., and also the structure of the isolated colonies -- size, form, surface, structure, transparency, color, etc.)
   b. when injecting cultures into firm media (indicate medium, duration of growth, etc.)
   c. when implanting into liquid media (indicate media, etc.)
   d. when growing in anaerobic conditions (indicate media, etc.)
3. Biochemical properties: fermentation of carbohydrates, alcohols, and glucosides (indicate which).
   Formation of indole, hydrogen sulfide
   Hemolytic properties
   Other particulars.
4. Serological properties: agglutinative capability of serums (indicate type, series and type of serum, by whom prepared, the maximum dilution of serum at which the culture reacted, intensity of the reaction, etc.).

Nature of agglutination (O or N)

Special comments

5. Virulence: for which animals, in what doses, under what method of infection, by what material (live culture, its age, filtrate, lysate, etc.), number of animals on which virulence tested, what result (died, became reinfected, etc.).

6. Immunogenic properties: material for immunization (live culture, dead culture, etc.), method of introduction, dose, number of injections, duration of intervals between injections, kind of animal immunized, test results, intensity of immunity, name of culture used to infect, age of culture, dose, method of introducing, number of surviving animals.

7. Special data

   a. Method of storing culture

      Composition of special media

      Optimal storage temperature

      Subculturing time

   b. Cultivation conditions

   c. Medium used for obtaining toxin

   d. Other data

      Name of chief (head of live cultures museum), date, month, year.

FORM NO. 8

REGISTER OF CULTURES AND DISEASED ANIMALS MARKED FOR DESTRUCTION

(Record to be kept in the autoclave room)

Date	Quantity of infectious material (beakers, dishes, etc.) to be destroyed	Tank No.	Signature of persons delivering tank with infectious materials	Signature of person receiving tank with infectious materials
1	2	3	4	5

FORM NO. 9

OUTLINE OF DOCUMENT FOR DESTRUCTION OF GROUP I CULTURES

Date, month, year, we, the undersigned, chief or head of department (laboratory), institute (last name, first name, middle name), staff members of department or laboratory (last name, first name, middle name), destroyed by autoclaving (or other method) ---------- at ---------.

Document signed by: Chief or head of department (laboratory)

Staff member of department (laboratory).

FORM NO. 10

OUTLINE OF DOCUMENT FOR ACCEPTING DELIVERY OF GROUP I CULTURES

Date, month, year, we, the undersigned, chief or head of live cultures museum ----------

chief of special section ----------

hereby declare that ----------

(list in detail, the source of the shipment, documents accompanying

the shipment, whether accompanying documentation corresponds to labels on cultures, etc.), kind of packaging, condition in which cases and beakers arrived, contents of shipment, etc.

Document signed by: Chief or head of live cultures museum.

Chief of special section.

Staff member of live cultures museum.

Seal of the special section.

INSTRUCTIONS GOVERNING THE USE OF STATIONARY STEAM DISINFECTING CHAMBERS EMPLOYING STEAM, BOTH STEAM UNDER PRESSURE, AS WELL AS AT NORMAL ATMOSPHERIC PRESSURE.

(Confirmed on 8 October 1959 by the Main State Sanitation Inspector).

I. Basic regulations.

1. Wearing apparel, bedding, rags, brushes, and other materials which will not deteriorate form steam's action, are to be disinfected in the steam disinfecting chambers.

Steam chambers are also used for sterilizing cotton wadding, bandaging materials, and other items (bed sheets, robes, etc.).

2. Steam chambers employ as the active disinfecting agent saturated steam at 104-111 and 118-120 degrees, and also steam at normal atmospheric pressure (100 degrees).

3. In the interest of avoiding the deterioration of certain items, it is forbidden to subject leather, fur, rubber, velvet, silk, nylon, and glued materials, or materials containing leather, fur, etc. to the action of the steam chamber.

One should neither place materials and fabrics dyed with unstable dyes, nor print-fabrics, into the chamber, since they might lose their original coloring.

Note: Since wearing apparel and other woolen materials lose as much as 30 percent of their wear-life under the action of steam, it is recommended that they be cleansed in steam-formalin chambers. If these are not available, steam chambers set to normal atmospheric pressure (100 degrees) may be used.

4. Clothing and bed linen soiled by excretions (urine, feces, blood, pus, etc.) should not be disinfected in steam chambers, since the fabrics may become spotted. They should rather be disinfected in washing machines or by steeping in disinfecting solutions.

5. The steam chamber may be loaded with 50 kilograms of bedding for each cubic meter of the chamber's capacity.

The chamber, working at normal atmospheric pressure and at a temperature of 100 degrees, can accommodate 10-12 outfits (wearing apparel), each with an average weight of 6 kilograms (60-72 kilograms) for each square meter of usable space. The usable space in a (a) 2.76 cubic-meter Krupin Chamber is 1.3 square meters; (b) in a 1.5 cubic-meter Krupin Chamber — 0.9 square meters; and (c) in a 5 cubic-meter Rubner Chamber — 2.5 square meters.

III. Procedure for operating the chamber.

In disinfecting materials contaminated with especially dangerous infectious agents (anthrax, glanders, plague, cholera), the persons loading the chamber wear special garments (coveralls, cap, rubber boots, goggles, gauze respirator, and rubber gloves). When he is finished loading the chamber, the worker disinfects the surrounding area and places his special garments into the chamber, excepting the rubber boots, rubber gloves, and goggles, which are disinfected with a liquid.

INSTRUCTIONS GOVERNING THE USE OF STATIONARY STEAM-FORMALIN DISINFECTING CHAMBERS

(Confirmed on 8 October 1959 by the Main State Sanitation Inspector of the USSR).

I. Basic regulations.

1. Steam-formalin chambers disinfect materials through the use of steam-formalin and steam-air methods; disinfestation is performed through steam-air methods.

2. The steam-formalin method uses, as the active disinfecting agent, a mixture of air and steam combined with formaldehyde gas, at a temperature ranging between 42 and 59 degrees.

3. The active disinfecting and disinfesting agent in the steam-air method is hot, moist air (air and steam mixture). Disinfection is carried out at a temperature ranging between 80 and 97 degrees; disinfestation, at a temperation between 49-57 and 80 degrees.

4. The relative humidity for both methods must be not less than 80 percent. Two thermometers -- one wet-bulb and one dry-bulb (August psychrometer) -- should be used for determining relative humidity. By comparing the readings, the chamber's relative humidity can be determined. If the difference between the readings of the wet-bulb and dry-bulb thermometer does not exceed four degrees, the relative humidity may be considered to be within normal limits.

5. The steam-formalin method is used for disinfecting things which deteriorate at a temperature of 60 degrees, as measured by an outside thermometer: leather, fur, rubber materials, footwear, etc.

The steam-air method is used to disinfect materials which do not deteriorate at temperatures above 60 percent, according to outside temperature: wool, cotton, oilcloth, velvet, synthetic and natural silk (including nylon) and also blankets, pillows, and mattresses.

The disinfection of cotton and woolen goods is effected through the use of a steam-air mixture, applied at a temperature of 80-90 degrees; leather and fur materials are treated at temperatures from 49-51 and 57-59 degrees.

6. It is not recommended to disinfect body and bed linen, especially that which has been stained by excretions (urine, feces, pus, blood, etc.); these should be disinfected by steeping them in disinfectant solutions.

7. Materials to be disinfected should be sorted out according to the disinfecting method to be employed for each item.

9. Where the steam-formalin method is employed, materials are hung in the chamber on special hangers, which preclude the garments' touching; the steam-air method employs ordinary hangers.

The amount of wearing apparel — contaminated with vegetative and spore-forming microbes -- which can be loaded into the chamber ranges between 2½ and 5 outfits (with an average weight of 6 kilograms each), or from 15 to 30 kilograms for each square meter of usable chamber space.

When the chamber is loaded with both wearing apparel and bed linen (mixed load), the loading norms for one square meter of usable

chamber space apply.

In a mixed load:

(a) bed linen is hung in a checkerboard arrangement;

(b) mattresses and feather-beds are hung in the chamber on wooden laths or are suspended from hooks; pillows are tied to the hooks with the aid of a string attached to one of the pillow's corners;

(c) children's apparel is hung in two rows; standard hooks, as well as long hooks (40-60 centimeters), are used.

10. When the items are contaminated by vegetative and spore-forming microbes, the loading norms for the steam-air method vary from 8 to 10 outfits, or from 48-60 kilograms for each square meter of usable chamber space.

PROCEDURE FOR OPERATING STATIONARY STEAM-FORMALIN CHAMBERS

A. Disinfection through the use of the steam-formalin method.

Preparing and loading the chamber:

12. Before operation, the steam pipes and steam jets in the chamber must be checked. The chamber is then heated to a temperature of 60 degrees (according to an external thermometer) by means of steam emitted from perforated pipes; this temperature is maintained for 15 minutes. The chamber is then aired out and loaded with materials.

> Note: The action of the jets should be periodically tested with water. For a more accurate determination of the jets' atomizing power, the water used in the test should be tinted and a sheet of white paper suspended on the opposite wall of the chamber. The quality of atomization is the test of the working order of the jets.

13. Items to be disinfected are placed in the chamber on special hangers; fur-lined items are turned inside out.

Felt and leather boots are hung up with the tops down; shoes and slippers are placed in nets or else hung on strings or hooks.

14. Disinfection is carried out at varying temperatures (57-59 or 49-51 degrees), depending on the materials. A less severe temperature is used for disinfecting fur (gopher and rabbit fur), which deteriorates at these temperatures.

Disinfection of materials contaminated with sporozoic microbes.

26. Disinfection is carried out at temperatures of 57-59 degrees; the loading norms are three outfits for each square meter of

THE STEAM-FORMALIN METHOD OF DISINFECTING FOOTWEAR AND LEATHER, FUR AND RUBBER ARTICLES

Kind of contamination	Load per sp. meter of usable chamber space		Temperature on outside thermometer	Formalin expended (milliliters) per cubic meter of space	Exposure time (minutes)	Overall operation time (minutes) not counting loading and unloading
	No. of outfits	Kilograms				
Sporous microbic forms	3	18	57-59	250	165	215

Method of disinfecting and kind of microbe	No. of outfits	Loading norms for bedding in place of work clothes (outfits)			
		Mattresses	Pillows	Blankets	
				Wool	Cotton
Steam-formalin (sporous forms)	3	2	3-4	5	3-4

usable chamber space; 250 milliliters of formalin is normally used for each cubic meter of chamber space. Exposure time is two hours and forty-five minutes. The items are loaded into the chamber in accordance with paragraph 13.

Note: In disinfecting sheepskin coats, it is recommended that rods 65 centimers long be placed in the sleeves to straighten them out and to prevent their touching the fur. The items should be hung in a checkerboard arrangement.

27. When the chamber is loaded and the doors closed, the steam is turned on slowly, and the chamber is heated to 53 degrees. Then the formalin is atomized, and the chamber temperature is raised to 57-59 degrees (beginning of exposure). Throughout the period of exposure, chamber temperature is kept at 57-59 degrees. At the end of the exposure, the formaldehyde is neutralized.

B. Disinfection through the use of the steam-air method; disinfection of items contaminated with sporozoic microbes and dermatophytes.

32. The disinfection of items contaminated with sporozoic microbes and dermatophytes is carried out at a temperature of 97-98 degrees. The loading norm is 10 outfits per square meter of usable chamber space. The exposure time is 30 minutes. After the chamber is loaded, steam heats the chamber to 97-98 degrees. The exposure then begins.

33. Steam is regularly supplied to the chamber so that a

DISINFECTING COTTON, WOOLEN, LEATHER, AND FUR ARTICLES WITH A STEAM-AIR MIXTURE

Kind of contamination	Method of decontamination	Loading norms per square meter of chamber space		Temperature on outside thermometer	Exposure (minutes)	Overall processing time, in minutes, not counting loading and unloading
		Outfits	Kilograms			
Sporous microbic forms	Disinfection	10	60	97-98	30	70-85

constant temperature of 97-98 degrees is maintained throughout the exposure. At the end of the exposure time, the steam valve is turned off, and the chamber is aired out for 10-15 minutes, after which the items are removed.

III. Procedure for operating the stationary chamber.

43. Persons engaged in the disinfecting of items contaminated with especially dangerous infectious agents (anthrax, glanders, plague, cholera) must wear special clothing (coveralls, rubber gloves, boots, respirator, cap, goggles). When they are finished loading the chamber, they apply a liquid disinfectant to the work area and place all their special clothing into the chamber.

TEMPORARY INSTRUCTIONS FOR DISINFECTING RAGS IN STEAM CHAMBERS

(Approved on 1 August 1951 by the Chief Sanitation-Antiepidemic Administration of the Ministry of Health USSR).

1. All rags must be suspected of possible carriers of anthrax.

2. Rags should be disinfected in steam chambers at a pressure of not less than 0.5 atmospheres.

3. The loading norm is up to 80 kilograms for each cubic meter of space in the chamber.

4. Rags should be placed loose into the chamber. Bales of rags may be placed in the chamber if the stay ropes are then cut. One level of bales may be placed in the chamber; they may not be piled one on top of another.

5. When the chamber is loaded and the doors closed, steam is slowly released into the chamber. In order to gradually force the air out of the chamber, the steam-exhaust valve is opened all the way.

6. When the thermometer connected to the steam-exhaust pipe shows 100 degrees (the air has been exhausted from the chamber), the steam-exhaust valve is partly closed and the steam-inlet valve is opened all the way. This causes the pressure in the chamber to rise to 0.5 atmospheres. At the same time, the readings of the thermometers mounted on the chamber will show a temperature rise.

7. Not until the thermometer mounted on the steam-exhaust pipe registers 110-111 degrees does the exposure period commence. Exposure is maintained at this temperature for 1½ hours. During this

period, chamber temperature and pressure is regulated by the steam-exhaust and steam-inlet valves.

8. During the operation, condensation is released from the chamber by means of a condensation-release valve mounted on the steam-exhaust pipe.

9. At the end of the exposure period, steam supply to the chamber is stopped. In order to bring the chamber's pressure down to zero, the steam-exhaust valve is opened all the way and a fan is turned on.

10. When the chamber pressure reaches zero, compressed air is fed into the chamber and the chamber is aired out.

11. The airing out and drying of the rags is done in 20-25 minutes, following which the valve supplying steam to the ejector is turned off. The door to the unloading (clean) side of the chamber is opened and the rags loaded onto a cart.

INSTRUCTIONS ON INVESTIGATING DRINKING WATER FOR AGENTS CAUSING TYPHUS ABDOMINALIS, PARATYPHOID FEVER, DYSENTERY, CHOLERA AND ANTHRAX.

(Confirmed on 27 June 1955 by the Ministry of Health of the USSR)

(Extract)

I. General regulations.

A. Selection, storage and transporting of samples.

1. Water samples to be tested for the presence of pathogenic microbes must be taken by specially instructed laboratory workers.

2. Water samples are poured into sterilized vials equipped with ground stoppers. The sterilization of the vials and stoppers is carried out as two separate operations. The vial is then corked tightly with a cotton stopper, and the ground stoppers are wrapped in a paper cap and tied to the necks of the vials.

If vials with ground stoppers are not available, water samples are taken in carefully washed bottles. These bottles must be tightly corked with cotton and a paper cap placed on top of the cotton and tied to the neck with twine.

3. Receptacles used for taking water samples must first be sterilized for an hour with dry heat at 160-170 degrees.

4. Before taking the sample, the water tap or hydrant from which the water is to be taken is first seared with flame. Then about thirty liters of water is allowed to escape. The sample for analysis is taken while the water is still running.

5. In taking water samples from water taps or water pipes, receptacles are filled in the following fashion: the twine holding the paper cap is untied, and the cotton stopper is grasped and removed with the aid of the paper cap. In taking the sample, care is exercised to see that the mouth of the bottle does not touch the tap or pipe. The bottle should be held at an angle and filled to four-fifths of its capacity. The stream of water should be regulated so as to prevent splashing.

6. When the bottle is full, it should be corked with the ground stopper or cotton stopper and the paper cap tied to the neck of the bottle with twine.

7. In taking water samples from wells, a bucket may be employed if there is no pump. The most sanitary method of extracting the sample is to use a weighted, sterilized receptacle. The receptacle, wrapped in paper, or gauze and paper, should be sterilized together with the twine and weight. The sterilized receptacle should be unwrapped just before taking the sample.

The uncorked sterilized receptacle is lowered into the well while the sample-taker holds the cotton stopper protected by its paper cap.

8. The water samples should be between one and three liters, but not less than 500 milliliters.

In testing for the presence of anthrax bacilli, three-liter water samples are used. In drawing samples from lakes and

reservoirs, 100 milliliters of silt, taken from different places, must be gathered from the lake or reservoir bottom. This silt is gathered in a separate sterilized receptacle.

9. The following data should be entered in the laboratory log:

(a) source of water (well, artesian well, water main)

(b) location of source from which sample was taken

(c) source of sample: water hydrant, water tap connected to main supplying water to residential area, nozzle, water tap of an area supplied from a water tank; tap of network supplied by reservoir; trough or pipe of a well pump; well shaft, etc.

(d) sample-taker (name, specialty)

(e) test target

(f) date and hour of taking water samples and date and hour samples were delivered to the laboratory.

10. In transporting water samples during the summer, care must be taken to protect the samples from heat and the action of the sun's rays; in the winter, they must be protected against freezing.

If ground stoppers are not available for transporting water samples intended for bacteriological analysis, the cotton stoppers must be kept from getting wet.

B. Preparations for analysis.

11. Testing of water samples for anthrax bacteria is carried out with the aid of No. 3 membrane filters.

Preparation of the equipment.

12. When dry, the membrane filters are highly inflammable and burn up very quickly; when wet, they present no danger whatsoever. If there is a large quantity of closely packed membrane filters on hand, they should be kept in a 20 percent alcohol solution. If, however, there are but a few of these filters, or if they are located in different places, they can be stored dry.

Brief (up to one year) storage of membrane filters in a dry state does not bring about any change in their properties or structure.

13. Membrane filters are sterilized for thirty minutes in distilled water, to which four or five drops of formalin have been added. After the first 15 minutes of boiling, water is poured off and fresh distilled water (without formalin) is added. The filters are then boiled for another 15 minutes. This operation is repeated twice. When sterilization has been completed, the membrane filters remain in the same water until they are used.

Filtration of the water.

14. After sterilization, the membrane filters are placed -- with the aid of sterilized and cooled pincers -- into a Zeytts Apparatus or an apparatus of the sort used by the Rublevskaya Pumping Station of the Moscow Water System, for filtration.

This apparatus is first sterilized in an autoclave, or burned with alcohol, or boiled in a closed vessel.

15. If a Zeytts filter is used, a sterile circular piece of filter paper must be placed on the metal screen to protect the membrane filters. These protective pieces are cut to the size of the membrane filter and sterilized in batches of 10 and 15. They are placed on the middle rack of the drying cabinet and sterilized by exposure to dry heat at a temperature of 160 degrees. Before use, the circular piece is picked with sterilized pincers and placed on the stage of the Zeytts Apparatus — all measures on sterilization being observed.

16. A sterilized, smooth-tipped pincer is used to place the membrane filter on the stage of the filtering device. It is important that all edges of the membrane filter lie flat on the metal stage. Then the upper part of the device is placed on top of the filter and secured tightly with metal clamps. The proper amount of water to be analyzed is then poured into the funnel of the Rublevskaya apparatus or into the Zeytts cylinder and the vacuum pump is started.

Note: When working in the field, or in the absence of an oil pump or water pump, a bicycle pump may be adapted for pumping out air. A tight clamp is fitted to the rubber stopper when the first drops of the filtrate appear.

17. In order to prevent the filters from becoming stopped up, the filtering is done fractionally through several membrane filters; depending on the rate of filtration, 25-50 milliliters of water is passed through each filter. After each filtration, the pump is turned off. To isolate pathogenic materials, a minimum of 500

milliliters of water must be filtered.

18. Upon completing filtration of the water, the upper part of the device is removed and the membrane filter is placed — with the aid of sterilized pincers — in the nutritive medium in such a way that the surface with the trapped microorganisms faces upward. The filter is carefully placed in the medium, starting with the free side and ending with the side held by the pincers.

Slight pressure in placing the membrane on the medium is permitted so as to preclude the possibility of air bubbles between the membrane filter and the medium. A normal-size Petri dish will accommodate four filters.

19. When working with water containing suspended particles, the water must first be filtered through a coarse membrane filter (plankton) and then filtered through No. 3 membrane filters. Plankton filters are also placed in the nutrient media. In both instances, the filtrate is gathered with due regard for sterility. After filtration through a membrane filter, the culture is checked for sterility.

20. For checking on the sterility of the filtrate, one milliliter of filtrate is placed in the beaker containing bouillon. If the filtrate proves to be unsterile and negative results are obtained from the test for pathogenic microbes, it may be necessary to re-run the test.

II. The Analysis.

B. Test for Anthrax Bacilli.

Concentration of the bacteria and preparations for analysis.

39. Before running the test, the microbes should be concentrated in the greatest possible volume, which can be done in one of the following ways:

(1) one liter of the water to be tested is passed through No. 3 membrane filters; from 50 to 250 milliliters of water should be passed through each filter, depending on the rate of decrease in filtering capacity. Then, all filters should be placed in a vessel and 10-20 milliliters of water stirred in. Let these stand for 5-10 minutes and use the liquid for bacteriological analysis. If the water is opalescent or if it contains suspended particles, act in accordance with the instructions in paragraph 19.

(2) if it is not possible to make use of membrane filters, the Fikker settling-out method may be used for isolating anthrax bacilli.

Twelve milliliters of a 10 percent solution of crystallic sodium carbonate (Na_2CO_3) and 10.5 milliliters of a 10 percent solution of iron sulfate ($Fe_2(SO_4)_3$) are added to the water to be tested; both solutions must be sterile. The mixture is stirred and exposed to cold for one hour.

If, instead of settling out, a part of the crystals rise to the surface, the mixture must be stirred again and let stand for yet another hour. Then, the transparent layer of water is poured off carefully so as not to stir up the sediment. The sediment and

remaining water is poured into centrifugal beakers and centrifuged for five minutes. In pouring water from the beakers, the sediment is dissolved in a 25 percent sterile solution of neutral potassium tartrate ($C_4H_4O_6K_2$); this is added dropwise and stirred until the sediment dissolves.

Seeding and growing.

40. Sediment and material obtained from the concentration treatment of membrane filters is divided into three portions. The first portion is heated at 65-70 degrees for 30 minutes; the second is seeded in a sugar bouillon (one part sediment to five parts bouillon) after being heated for 15 minutes in hot water. The sugar bouillon seedings are covered with a thin layer of liquid vaseline and placed in a thermostatic enclosure at 37 degrees. Twenty-four hours later, they are heated 15 minutes at 80 degrees. The third portion of the sediment does not undergo any processing whatsoever.

In the first portion, all nonsporogenous forms die, leaving the sporogenous, including anthrax microbes.

In the second portion, anaerobic spores develop; in the sugar bouillon, these are transformed into vegetative forms. The next heat treatment destroys all nonsporogenous forms, as well as embryo sporogenous anaerobes; only sporogenous aerobes (and, occasionally, isolated anaerobes who have not yet developed spores) remain.

The third portion is used for seeding and infection purposes. It is not subjected to any processing, so as to preclude

any false conclusions arising from the deletion, during heating, of certain occasionally unstable forms of anthrax bacilli spores.

41. The first portion is subcultured on three Petri dishes containing agar and in three beakers containing bouillon; a third portion is seeded in five dishes containing agar and three beakers with bouillon. A spatula is used for the subculturing from one dish to the next. During this process, the spatula is not sterilized.

Reviewing the results of the analysis

42. After a lapse of 16-20 hours, the subcultures are examined for suspect colonies (soft, blue-white, harder in the center, with an observable fine-grained structure). These colonies are examined through a (third system) microscope. One is usually able to discern interwoven curly fibres resembling locks of wavy hair (the so-called "Medusa head"); the colonies are further examined in order to differentiate them from pseudoanthrax and anthracoids having the same structure as real anthrax bacilli.

The material from the colonies is first examined in a suspended droplet (anthrax bacilli are immobile, while pseudoanthrax bacilli in young cultures have a faint independent mobility).

Then subcultures are made on bloody agar containing 15-20 percent blood (anthrax colonies give the appearance of grey-yellowish compact colonies growing without blood hemolysis; pseudoanthrax and anthracoids hemolyze blood).

Biological test.

43. The final diagnosis is made on the basis of the death of mice who have been infected with material from the colonies; death occurs between 12 and 96 hours. Blood smears taken from the spleen and kidneys of the dead mice reveal articulated fibres with capsules.

44. White mice are infected with a suspension from each of the three portions. The material is injected subcutaneously into the back. The dead mice undergo a special examination.

Examination of the silt (sediment) for anthrax bacilli.

45. A thirty milliliter sample from the silt is agitated in 100 milliliters of water for five or ten minutes. Then, the suspensions are allowed to stand for 10 minutes. The liquid is then poured off and passed through membrane filters (if membrane filters are used) or the liquid is centrifuged; the material obtained from the filter or centrifuge is tested by the previously described method (paragraphs 40 and 41).

In addition, a 10 gram silt sample, which has undergone no processing, is analysed by the same method.

46. The limit of sensitivity of the method of analyzing using microbic concentrations and membrane filters is 200-300 spores to one liter. Maximum sensitivity under the Fikker spore concentration method is not known.

DIRECTIONS FOR THE VACCINATION OF HUMANS WITH ANTHRAX STI LIVE VACCINE BY SCARIFYING THE SKIN

(Approved on 25 May 1959 by the Committee on Vaccines and Serums of the Ministry of Health USSR)

1. Anthrax STI live vaccine is a suspension of spores of a culture obtained from anthrax vaccine strains. The vaccine is dispensed in sealed ampules.

2. The vaccine should be stored in a dark place at a temperature between 0 and 4 degrees. When the vaccine is stored under these conditions, it retains its effectiveness for two years. Through re-checking, the vaccine's usable period may be extended by two additional years. Re-checking should be carried out on vaccine series in a minimum quantity of 5000 doses.

3. Vaccination is contraindicated in acute infectious illness, in cases involving inadequate blood circulation, in active tuberculosis, in primary and advanced cachexia, in acute and chronic lymphadenitis, in acute and chronic illness of the kidneys, liver and central nervous system, in severe forms of endocrinal illness, in the second half of pregnancy, and in the case of toxicosis in any stage of pregnancy. Chronic malaria sufferers should be given quinine or quinacrine before vaccination and on the day of vaccination.

4. Vaccinations are administered:

(a) at enterprises engaged in the processing of animal products, especially leather and wool, and at meat packing plants.

(b) at collective and state farms where anthrax is reported (people handling livestock are vaccinated).

(c) in other cases, persons are vaccinated on the basis of epidemiological indications.

5. Vaccinations are administered by physicians.

6. Vaccinations are given one time. Booster vaccinations should be given a year later.

7. Before use, vaccine ampules should be carefully examined. If an ampule is cracked, or if there are foreign bodies in the vaccine, or if there is an imbalance in the suspension, the ampule containing the vaccine must be destroyed. Vaccine is to be used within one hour after the ampule is opened.

8. Before use, the ampule is to be agitated. The upper part of the ampule neck is cut, wiped with alcohol, and held over a flame. Care must be taken that the entire ampule containing the vaccine does not become heated. The neck of the ampule is broken off, and the vaccine is transfered by a pipette or long-needled syringe to a sterile vial. Vaccination is done with a sterile instrument (scarifier) by scarifying the skin of the upper arm. The skin is first rubbed with alcohol (no iodine, mercuric chloride, or other disinfecting solutions are used), and then with ether to remove grease. When the ether has evaporated, two drops of vaccine are placed 3-4 centimeters apart on the skin with a sterilized eye dropper. In administering the vaccine, care must be taken not to let the dropper touch

the skin. The vaccine must be administered so as to form a spot approximately 1.5 centimeters in diameter and must not drip from the arm. The physician then grasps the underside of the patient's upper arm and draws the skin taut. Holding the scarifier in his right hand, he makes four or five scratches over the area treated with vaccine. The scratches should be superficial, with tiny droplets of blood appearing in the scratch furrows. Thereupon, the vaccine is rubbed in with the flat surface of the scarifier for one or two minutes.

After the vaccination, the vaccine must be allowed to dry for five or ten minutes. Then the remnants are removed with a sterile cotton tampon, after which the patient may get dressed.

The used scarifier is then boiled before the next vaccination.

9. As a rule, there is no general reaction to the vaccination. The surrounding lymph nodes do not become enlarged. A local reaction occurs in the form of a reddening of the scratched area and a scab which quickly drops off.

10. A medical check is maintained, according to the general rules, for persons vaccinated. A vaccination log is maintained, together with a register of post-vaccination examinations. In this register, the name of the vaccine, the series number, the method of administering, and the date of vaccination are recorded.

11. Vaccine remnants and instruments used in vaccination are then boiled for two hours.

DIRECTIONS FOR SUBCUTANEOUS VACCINATION OF HUMANS WITH ANTHRAX STI LIVE VACCINE

(Approved on 25 May 1959 by the Committee on Vaccines and Serums of the Ministry of Health USSR)

1. Anthrax STI live vaccine is a suspension of spores of a culture obtained from anthrax vaccine strains. The vaccine is dispensed in sealed ampules.

2. The vaccine should be stored in a dark place at a temperature between 0 and 4 degrees. When the vaccine is stored under these conditions, it retains its effectiveness for two years. Through re-checking, the vaccine's usable period may be extended by two additional years. Re-checking should be carried out on vaccine series in a minimum quantity of 5000 doses.

3. Vaccination is contraindicated in acute infectious illness, in cases involving inadequate blood circulation, in active tuberculosis, in primary and advanced cachexia, in acute and chronic lymphadenitis, in acute and chronic illness of the kidneys, liver and central nervous system, in severe forms of endocrinal illness, in the second half of pregnancy, and in the case of toxicosis in any stage of pregnancy. Chronic malaria sufferers should be given quinine or quinacrine before vaccination and on the day of vaccination.

4. Vaccinations are administered:

(a) at enterprises engaged in the processing of animal products, especially leather and wool, and at meat packing plants.

(b) at collective and state farms where anthrax is reported (people handling livestock are vaccinated).

(c) in other cases, persons are vaccinated on the basis of epidemiological indications.

5. Vaccinations are administered by physicians.

6. Vaccinations are given one time. Booster vaccinations should be given a year later.

7. Before use, vaccine ampules should be carefully examined. If an ampule is cracked, or if there are foreign bodies in the vaccine, or if there is an imbalance in the suspension, the ampule containing the vaccine must be destroyed. Vaccine is to be used within one hour after the ampule is opened.

8. Before use, the ampule is to be agitated. The upper part of the ampule neck is cut, wiped with alcohol, and held over a flame. Care must be taken that the entire ampule containing the vaccine does not become heated. The neck of the ampule is broken off, and the vaccine is transferred by a pipette or long-needled syringe to a sterile vial.

The vaccine is injected in 1 milliliter quantities -- with proper regard to asepsis -- subcutaneously, under the lower edge of the scapula of the back. Children up to the age of ten are given 0.3 milliliters; from eleven to fourteen, 0.5 milliliters. The vaccination point is swabbed with alcohol and a little tincture of iodine is applied before the vaccine is injected. After the injection,

tincture of iodine is again applied to the vaccination point. Needles and syringes are sterilized by boiling, exclusively. A different needle is used for each vaccination.

9. In the overwhelming majority of cases, no general reaction is observed whatsoever. The surrounding lymph nodes do not increase in size. In isolated cases, there may be some signs of a general reaction: malaise, headache, slight feeling of weakness and a slight rise in temperature. All these disappear in two or three days. Local reaction is seldom seen. When it occurs, it takes the form of redness, pastosus, and painfulness in the area where the vaccine was injected. Local reaction lasts between two and four days. In rare instances, the surrounding lymph nodes become enlarged. All these post-vaccination features pass quickly, without consequence.

10. A medical check is maintained, according to the general rules, for persons vaccinated. A vaccination log is maintained, together with a register of post-vaccination examinations. In this register, the name of the vaccine, the series number, the method of administering, and the date of vaccination are recorded.

11. Vaccine remnants and instruments used in vaccination are then boiled for two hours.

DIRECTIONS FOR DIAGNOSING ANTHRAX AND FOR DETERMINING POST-INFECTION AND POST-VACCINATION REACTIVITY TO IT THROUGH AN INTRACUTANEOUS ALLERGY TEST EMPLOYING THE ANTHRAX ALLERGEN, "ANTHRAXIN".

(Approved on 20 February 1960 by the Committee on Vaccines and Serums of the Ministry of Health USSR).

The method of diagnosing anthrax using the anthrax allergen "anthraxin" is based on the specific ability of the macroorganism of one afflicted with anthrax, one who has had anthrax, or been vaccinated against anthrax, to respond with a local allergic reaction in the form of hyperemia and infiltration, when the allergen is intracutaneously injected. This ability develops already in the first days of the illness, or in the first few days after vaccination, and is retained for a number of years by those who have had anthrax.

Method for conducting the intracutaneous test.

The injection is made -- with due regard to the rules on asepsis -- on the underside of the forearm. Anthraxin is administered intercutaneously with the aid of a syringe. The dose is from 0.05 to 0.1 milliliter.

The other forearm is injected with a like dose of a test-control liquid. This is done using another syringe equipped with a fine needle.

Evaluation of the results.

In positive cases, hyperemia and the beginning of infiltration is observed in between six and ten hours in the area where the anthraxin was injected. Within 24 hours, the reaction takes the form of clearly visible changes in the dimensions of the area of hyperemia

and skin infiltration. The reaction is observed from 24 to 48 hours.

The use of the following table is recommended for evaluating the degree of the reaction:

Local reaction features

After 24 hours	After 48 hours	Evaluation
Reaction absent, or hyperemia not accompanied by infiltration.	No reaction.	Negative (-)
Hyperemia and infiltration	Reaction completely ended.	Questionable (±)
Hyperemia without infiltration, or hyperemia up to 15 millimeters in diameter with infiltration.	Hyperemia (possible infiltration)	Slightly positive (+)
Hyperemia 16-25 millimeters in diameter with infiltration.	Hyperemia (possible infiltration).	Positive (++)
Hyperemia 26-50 millimeters in diameter with infiltration.	Hyperemia (possible infiltration)	Positive (+++)
Hyperemia more than 51 millimeters in diameter with infiltration.	Hyperemia (possible infiltration)	Very positive (++++)

Note: 1. Hyperemia and infiltration are considered only if they are more than 5 millimeters in diameter.

2. Development of hyperemia and infiltration in the area injected with the test control liquid justifies the cancelling of the test. The usable period for anthraxin stored in a dry, dark place at four and ten degrees is one year from the date of preparation.

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